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REVIEW ARTICLE

T-Cell Dysfunction and Inhibitory Receptors in Hepatitis C Virus Infection

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Dysfunction of the virus-specific T cells is a cardinal feature in chronic persistent viral infections such as one caused by hepatitis C virus (HCV). In chronic HCV infection, virus-specific dysfunctional CD8 T cells often overexpress various inhibitory receptors. Programmed cell death 1 (PD-1) was the first among these inhibitory receptors that were identified to be overexpressed in functionally impaired T cells. The roles of other inhibitory receptors such as cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and T cell immunoglobulin and mucin domain-containing molecule 3 (Tim-3) have also been demonstrated in T-cell dysfunctions that occur in chronic HCV patients. Blocking these inhibitory receptors in vitro restores the functions of HCV-specific CD8 T cells and allows enhanced proliferation, cytolytic activity and cytokine production. Therefore, the blockade of the inhibitory receptors is considered as a novel strategy for the treatment of chronic HCV infection.
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INTRODUCTION

Hepatitis C virus (HCV) is a positive-sense single-stranded RNA virus of the genus *Hepacivirus* in the family *Flaviviridae*, and it infects 170 million people worldwide (1). About 10～60% of the patients clear HCV spontaneously during the acute phase of the infection (2), while the others develop chronic persistent HCV infection that eventually leads to liver cirrhosis and hepatocellular carcinoma (3). Spontaneous resolution of HCV infection correlates with robust and sustained responses of the virus-specific T cells as demonstrated in humans (4-6) and in chimpanzees (7,8), the sole animal model of HCV infection. On the other hand, the progression towards chronic HCV infection is associated with weak and transient responses of the virus-specific T cells (4-8). Various dysfunctions of the HCV-specific T cells, such as inefficient proliferation, cytolytic activity, and cytokine production, are commonly observed during the chronic stage of HCV infection (reviewed in 9,10). Impaired cellular immune responses have been attributed to the mutations within the T-cell epitopes (11-13), a deviated differentiation of T cells (14) and suppressive functions of the regulatory T cells (15). Dysfunctional T cells are also observed in other chronic persistent viral infections such as hepatitis B virus (HBV), human immunodeficiency virus (HIV) in humans, and lymphocytic choriomeningitis virus (LCMV) infection in mice (16).

A novel mechanism of T-cell dysfunction was recently demonstrated in a murine model of chronic LCMV infection (17). It was found that the expression of programmed cell death 1 (PD-1) was up-regulated on dysfunctional LCMV-specific CD8 T cells in mice (17). In vivo blockade of the interaction between PD-1 and its ligand, PD-L1, restored the functions of LCMV-specific CD8 T cells and reduced the viral titer (17). This influential discovery led to extensive investigations of the role of PD-1 in the regulation of T cells in human chronic viral infections (16). More recently, other inhibitory receptors such as cytotoxic T lymphocyte asoociated antigen 4 (CTLA-4) and T cell immunoglobulin and mucin domain containing molecule 3 (Tim-3) have also been studied as the factors that can cause T-cell impairments in chronic viral infections. In this review, the roles of various inhibitory receptors in T-cell dysfunction found in chronic HCV infection

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are summarized.

THE ROLE OF PD-1 IN HCV INFECTION

PD-1 is one of the inhibitory receptors which are expressed on the T cells. It has two known ligands, PD-L1 and PD-L2, which are members of B7 family. Upon binding to its ligands, PD-1 confers inhibitory signal to the T cells by recruiting SH2-containing phosphatases, SHP-1 and SHP-2, to its immunoreceptor tyrosine-based switch motif (ITSM). Recruited phospatases then block the T-cell receptor (TCR)-mediated activatory signal at proximal site (16).

The role of PD-1 in virus-specific T cells in chronic viral infections was first identified in a murine model of chronic LCMV infection (17). As in chronic LCMV infection, the expression of PD-1 is similarly upregulated on the virus-specific CD8 T cells in chronic HCV infection, and HCV-specific PD-1^{high} T cells are functionally impaired (18-20). In addition, a blockade of PD-1/PD-L1 interaction restores T-cell functions such as proliferation, cytolytic activity and cytokine (IFN-γ and TNF-α) production (18-20). The PD-1^{high} dysfunctional CD8 T cells express low levels of CD127, a marker of memory precursors, and high levels of CD57, a T-cell senescence marker (18,20). PD-1^{high}CD127^{low} HCV-specific CD8 T cells are known to frequently undergo apoptosis (21). PD-1 expression is likely to be influenced by the location of HCV-specific CD8 T cells in vivo, since HCV-specific CD8 T cells in the liver have a tendency to express higher levels of PD-1 than those found in the peripheral blood (22). In addition, PD-1/PD-L1 blockade was able to functionally restore HCV-specific CD8 T cells originating from the peripheral blood, but not those found in the liver (22). The dissimilarities among the virus-specific CD8 T cells found in different in vivo compartments need to be considered in further studies.

The role of PD-1 was also studied in the acute stage of HCV infection. Specifically, the relationship between the PD-1 expression and the outcome of the acute HCV infection was questioned. Recent studies showed that the progression of acute HCV infection to the chronic stage is associated with a high level of PD-1 on HCV-specific CD8 T cells during the acute infection, and the clearance of HCV infection is associated with lower levels of PD-1 expression (23,24). However, at least one other study reported that the high level of PD-1 during the acute HCV infection is irrespective of the outcome of HCV infection (25). In a chimpanzee model of acute HCV infection, intrahepatic levels of PD-1 were determined by prospective liver biopsy and real-time PCR, and high mRNA levels of PD-1 were found to be associated with the development of chronic HCV infection (26). Intriguingly, the PD-1 levels in HCV-specific CD8 T cells declined through the escaping mutation of cognate T-cell epitopes even in the chronic stage of HCV infection (23). This implies that the high PD-1 levels on virus-specific CD8 T cells are maintained by persistent TCR stimulation, which henceforth explains why high PD-1 expression is routinely observed in chronic persistent viral infections.

Very recently, PD-1 expression was studied in the experimental vaccine trials and subsequent HCV challenge in chimpanzees (Shin et al., unpublished data). In this study, the phenotypes of HCV-specific CD8 T cells were analyzed in HCV-challenged chimpanzees, which were part of a previously published adenovirus/DNA-based HCV NS3-NS5 vaccine study (27). HCV-specific CD8 T cells from the vaccinated chimpanzees displayed lower levels of PD-1 and a greater ability to secrete IFN-γ than those from the control group. Consistent with these findings, intrahepatic mRNA levels of PD-1 and PD-L1 were significantly lower in the vaccinated chimpanzees than in the control chimpanzees. These data showed that the low expressions of PD-1 and PD-L1 are characteristic features of vaccine-induced resolution of acute HCV infection, and that the attenuation of the PD-1/PD-L1 inhibitory pathway during vaccine-induced HCV clearance may enable HCV-specific CD8 T cells to have enhanced anti-viral functions.

THE ROLE OF CTLA-4 IN HCV INFECTION

CTLA-4 is structurally homologous to CD28, an important T-cell costimulatory molecule, and its expression is upregulated on the activated T cells (28). CTLA-4 exerts T-cell inhibitory functions through diverse mechanisms. CTLA-4 binds to CD80 and CD86, thus competitively inhibiting the interaction between CD28 and B7 molecules. In addition, CTLA-4 recruits phosphatases such as SHP-2 and blocks the signal activated by TCR ligation (28).

Although the blockade of CTLA-4 did not result in a restoration of the T cell functions in chronic LCMV infection in the previous study (17), recent findings on CTLA-4's role in chronic HCV infection showed promising results (22,29). The HCV-specific CD8 T cells found in the livers of chronic HCV patients did not only overexpress PD-1, but also

CTLA-4. Co-expression of PD-1 and CTLA-4 was observed in liver-infiltrating lymphocytes, but not in peripheral blood lymphocytes (29), suggesting the phenotypic differences of virus-specific CD8 T cells in different in vivo compartments. PD-1$^+$CTLA-4$^+$ HCV-specific T cells were profoundly dysfunctional (22). The functions of PD-1$^+$CTLA-4$^+$ HCV-specific CD8 T cells could be restored by a combined blockade of PD-1 and CTLA-4, but not by PD-1 blockade or CTLA-4 blockade alone (29). For the development of a novel therapeutic strategy to restore the functions of HCV-specific CD8 T cells, a combined blockade of multiple inhibitory receptors needs to be done in order to maximize the anti-viral functions of HCV-specific CD8 T cells.

THE ROLE OF Tim-3 IN HCV INFECTION

Tim-3 was originally discovered as a specific marker of Th1 CD4 T cells (30). It has been known that interaction of Tim-3 with its ligand, galectin-9, promotes the cell death of Th1 cells and terminates Th1 responses.

The role of Tim-3 in chronic viral infections was first identified in HIV infection (31). In HIV-infected patients, the frequency of Tim-3$^+$ CD8 T cells increased, and the Tim-3 levels on the T cells correlated positively with the viral titer and inversely with CD4 T cell count (31). The HIV-specific CD8 T cells that overexpress Tim 3 were found to be functionally impaired, and a blockade of Tim-3 restored the functions of HIV-specific CD8 T cells (31).

After this finding, the role of Tim-3 has also been studied in chronic HCV infection (32). Tim-3 is over-expressed on HCV-specific dysfunctional CD8 T cells, and Tim-3$^+$ CD8 T cells are of CD127^{low}CD57^{high}, phenotype which is identical to that of PD-1$^+$ CD8 T cells in chronic HCV infection (18,20). Tim-3$^+$PD-1$^+$ HCV-specific CD8 T cells were preferentially enriched in the intrahepatic compartment over the peripheral blood. Importantly, a blockade of Tim-3 resulted in a functional restoration of HCV-specific CD8 T cells, evidenced by increased proliferation and IFN-γ production (32). The role of Tim-3 was also studied in HCV/HIV co-infection (33). Compared to HCV infection alone, the frequency of Tim-3$^+$PD-1$^+$ HCV-specific CD8 T cells was higher in HCV/HIV co-infection, and Tim-3/PD-1 co-expression correlated with liver damage (33). Either a Tim-3 blockade or a PD-1 blockade alone was found to be sufficient in restoring the functions of Tim-3$^+$PD-1$^+$ HCV-specific CD8 T cells. Interestingly, Tim-3$^+$PD-1$^+$ phenotype was more frequent in HCV-specific CD8 T cells than in HIV-specific CD8 T cells, implying the varying degrees of impairments in different virus specific T cells.

THE ROLE OF OTHER INHIBITORY RECEPTORS IN HCV INFECTION

In order to discover the other possible molecules that can potentially downregulate T-cell functions in chronic viral infections, microarray and gene expression profiling were performed in a murine model of LCMV infection (34). Several candidate molecules have been identified, including PD-1, lymphocyte activation gene-3 (LAG-3), 2B4, CD160, CTLA-4, paired immunoglobulin-like receptor B (PIR-B) and GP49B (34). A subsequent study demonstrated the complicated expression patterns of the inhibitory molecules and showed that the co-expression of multiple inhibitory molecules is associated with the severity of the infection (35). Very recently, co-expression of PD-1, 2B4, CD160, killer cell lectin-like receptor G1 (KLRG1), LAG-3 and CTLA-4 on CD8 T cells was studied in chronic HCV infection (36). Co-expression of multiple inhibitory receptors was observed on HCV-specific CD8 T cells and was associated with low levels of CD127 (36).

CONCLUSION

Since the discovery of PD-1 as an inhibitory receptor associated with T-cell dysfunction in chronic LCMV infection, the roles of various inhibitory receptors on virus-specific CD8 T cells have been extensively studied in human chronic viral infections such as HCV, HBV and HIV infections. As blocking the inhibitory receptors in vitro restored the functions of virus-specific T cells, the blockade has been considered as a novel strategy for the treatment of chronic viral infections (37). A recent study evaluated the in vivo blocking effects of anti-PD-1 antibody in macaques infected with simian immunodeficiency virus (SIV) (38). The PD-1 blockade was able to enhance the immune responses and resulted in a significant reduction of viral load and prolonged survival of the infected hosts (38).

However, for an in vivo blockade of the inhibitory receptors to be used in an actual therapy, some possible side effects must be considered. One study found that the infection of PD-L1-/- mice with a chronic LCMV strain was lethal due to severe immunopathologic damage (17), implying the importance of PD-1/PD-L1 in the prevention of virus-in-

duced lethal immunopathology. In particular, liver damage is known to be mediated by T-cell responses in HCV infections, and hence T cell-mediated liver damage may be aggravated by blockades of inhibitory receptors, resulting in lethal hepatitis (39).

As mentioned before, some patients recover from acute HCV infection and exhibit low level of inhibitory receptors, while the others enter the chronic stage of HCV infection that results in upregulation of inhibitory receptors and progressive loss of the T-cell functions. Thus, an important question that arises is the mechanism that influences the body's immune system to choose between those two directions of disease outcome during the acute HCV infection. Perhaps it could be based on the patient's genetic backgrounds, environmental influences, or both. In any case, the potential possibility that the blockade of the inhibitory molecules early on during the acute HCV infection may prevent the disease from progressing toward a more problematic chronic stage should be considered.

The restoration of the patients' own anti-viral immune functions has been pursued as a possible therapy for chronic HCV infection, but all efforts have been unsuccessful to date. The recent advancements in the understandings of the roles of the inhibitory receptors in T-cell dysfunction will hopefully aid greatly in the development of a highly effective therapy for HCV infection.

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CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

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